Antibiotic Transport via Runoff and Soil Loss

J. G. Davis,* C. C. Truman, S. C. Kim, J. C. Ascough II, and K. Carlson

ABSTRACT

Research has verified the occurrence of veterinary antibiotics in manure, agricultural fields, and surface water bodies, yet little research has evaluated antibiotic runoff from agricultural fields. The objective of this study was to evaluate the potential for agricultural runoff to contribute antibiotics to surface water bodies in a worst-case scenario. Our hypothesis was that there would be significant differences in antibiotic concentrations, partitioning of losses between runoff and sediment, and pseudo-partitioning coefficients (ratio of sediment concentration to runoff concentration) among antibiotics. An antibiotic solution including tetracycline (TC), chlortetracycline (CTC), sulfathiazole (STZ), sulfamethazine (SMZ), erythromycin (ERY), tylosin (TYL), and monensin (MNS) was sprayed on the soil surface 1 h before rainfall simulation (average intensity = 60 mm h^{-1} for 1 h). Runoff samples were collected continuously and analyzed for aqueous and sediment antibiotic concentrations. MNS had the highest concentration in runoff, resulting in the highest absolute loss, although the amount of loss associated with sediment transport was <10%. ERY had the highest concentrations in sediment and had a relative loss associated with sediment >50%. TYL also had >50% relative loss associated with sediment, and its pseudo-partitioning coefficient (P-PC) was very high. The tetracyclines (TC and CTC) had very low aqueous concentrations and had the lowest absolute losses. If agricultural runoff is proven to result in development of resistance genes or toxicity to aquatic organisms, then erosion control practices could be used to reduce TC, ERY, and TYL losses leaving agricultural fields. Other methods will be needed to reduce transport of other antibiotics.

V ETERINARY ANTIBIOTICS have long been used for treating and protecting the health of animals and to promote growth and feed efficiency (Thiele-Bruhn, 2003). According to the Animal Health Institute's 2002 Market Sales Report, livestock producers in the United States use 11.2 million kg of antibiotics every year in the absence of disease for nontherapeutic purposes: about 4.7 million kg in hogs, 4.8 million kg in poultry, and 1.7 million kg in cattle (Mellon et al., 2001). The relatively high usage of veterinary antibiotics and their potential persistence and impact in the environment have led to an interest in the fate and transport of these compounds. Major pathways for antibiotic transport into the environment include seepage from manure stor-

age facilities or runoff from fields fertilized with manure (Boxall et al., 2003). Although detailed transport mechanisms have not been studied, previous research has verified the occurrence of veterinary antibiotics in manure, agricultural fields, and water resources (Campagnolo et al., 2002; Haller et al., 2002; Hamscher et al., 2002; Schlusener et al., 2003; Yang and Carlson, 2003).

Antibiotics in the environment may lead to the development of antibiotic resistance, a critical concern as it relates to the efficacy of antibiotics in the treatment of human disease (Boxall et al., 2003). In addition to human health concerns, there are possible toxic effects of antibiotics on aquatic and edaphic organisms. Although most measured concentrations have been well below (an order of magnitude lower) levels that have been shown to be toxic to standard testing organisms, degradation products and interactions among compounds have not been adequately evaluated and could result in synergistic toxic effects (Boxall et al., 2003).

Sulfonamides, including sulfamethazine (SMZ) and sulfathiazole (STZ), have been detected at concentrations as high as 20 mg kg^{-1} in liquid manure samples (Haller et al., 2002). Macrolides and ionophore polyether groups were measured in liquid manure, and maximum concentrations were 43 μg tiamulin kg^{-1} and 11 μg salinomycin kg^{-1} (Schlusener et al., 2003). Campagnolo et al. (2002) measured five groups of antibiotic residuals, including tetracycline (TC), chlortetracycline (CTC), SMZ, and erythromycin (ERY) in animal waste, surface water, and groundwater resources near large-scale swine and poultry feeding operations. The compound with the highest concentration in lagoons was CTC with 1000 μg L^{-1}, followed by 400 μg L^{-1} of SMZ. Also, 31 and 67% of antibiotics tested were detected in water samples (surface water, tile drainage, and wells) taken near swine and poultry farms, respectively. This study indicates that animal waste stored in lagoons or applied to agricultural fields may be a source of veterinary antibiotics entering the environment.

Yang and Carlson (2003) measured tetracycline and sulfonamide levels in the Cache la Poudre River in northern Colorado; they reported no detectable concentrations in a pristine location in the mountains, but they did detect tetracyclines and sulfonamides in other sampling locations in both urban and agricultural areas. Another study measured residuals of tetracyclines and tylosin (TYL), which are widely used in livestock production, in soil fertilized with animal slurry (Hamscher et al., 2002). Average concentrations of 198.7 μg TC kg^{-1} and 4.6 to 7.3 μg CTC kg^{-1} at a soil

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Abbreviations: TC, tetracycline; CTC, chlortetracycline; STZ, sulfathiazole; SMZ, sulfamethazine; ERY, erythromycin; TYL, tylosin; MNS, monensin; OTC, oxytetracycline; LOQ, limit of quantification; P-PC, pseudo partitioning coefficient; HPLC, high performance liquid chromatography.

depth of 10 to 20 cm were found; tetracyclines were not detected below a 30-cm depth, and TYL was not detected in any soil or groundwater samples. This study confirmed that some antibiotics can enter the environment in significant concentrations, and antibiotic residues can accumulate in the soil when liquid manure is applied repeatedly.

Little research has been published that evaluates transport of veterinary antibiotics from agricultural fields to surface water. Addison (1984) developed a model to predict antibiotic transport in runoff and sediments from feedlots; however, the model was not tested with actual field data. A few recent studies have evaluated antibiotic leaching potential (Kay et al., 2005a, 2005b; Kreuzig and Höltge, 2005). Boxall et al. (2002) and Kay et al. (2004) have reported antibiotic leaching to tile drainage systems which then flow to surface water. Kay et al. (2005c) compared three antibiotics in their overland flow concentrations after "irrigation" with a knapsack sprayer. In this unreplicated study, the researchers detected sulfachloropyridazine and oxytetracycline (OTC) in runoff, but tylosin (TYL) was not detected. Burkhardt et al. (2005) reported sulfonamide concentrations in runoff from manured grassland following irrigation applied at the intensity of a heavy rainstorm. Kreuzig et al. (2005) compared sulfonamide transport by runoff from a tilled field to that from grassland and found that tillage reduced runoff volume and, hence, also reduced sulfonamide losses. Kreuzig et al. (2005) also used sprinkler irrigation to initiate runoff and study antibiotic transport, but based on their description, it appears that they used a single-nozzle rainfall simulator calibrated to be similar to rainfall in droplet size and kinetic energy. This is an important distinction because irrigation systems have different drop size distributions, and thus kinetic energies, compared to natural rainfall and rainfall simulations. More importantly, the distribution of water from irrigation systems is quite erratic compared to that of natural rainfall and simulations. Most irrigation systems apply water in "waves" so that after water hits the ground, there is a pause before the next pulse of irrigation water. This difference has a strong influence on runoff and sediment delivery (Römkens et al., 2002). A backpack sprayer does not apply water to the entire plot at the same time and, thus, introduces additional variance from natural rainfall. Our study is unique due to the use of rainfall simulation (not irrigation), the range of antibiotic chemistries evaluated (seven antibiotics from four classes), and the determination of sediment-to-solution partitioning of antibiotic losses.

Rainfall simulation experiments have been conducted to evaluate the transport of pesticides (Truman et al., 1998; Ma et al., 1999; Wesenbeeck et al., 2001; Li et al., 2002; Potter et al., 2003; Muller et al., 2004; Potter et al., 2004; Wauchope et al., 2004). In general, transport behavior of pesticides is mainly influenced by rainfall intensity, soil properties, and pesticide characteristics. Halling-Sørensen et al. (1998) concluded that since they have similar physicochemical properties, antibiotics are expected to have the same fate as pesticides if manure (containing antibiotics) is spread on fields just before a

rainfall event. Kay et al. (2004) agreed that processes governing pesticide fate also apply to antibiotics. Therefore, in this study, we utilized simulated rainfall in 6-m^2 plots, a methodology common to pesticide transport studies (Potter et al., 2003; Potter et al., 2004; Wauchope et al., 2004), to examine runoff, soil loss, and associated transport of seven selected veterinary antibiotics.

The basic chemical structure of the seven veterinary antibiotics evaluated is shown in Fig. 1 and 2. Tetracyclines are differentiated by various substitutions in the backbone (Lock et al., 1999; Bruno et al., 2002). Selected sulfonamide compounds have two N functional groups, and most sulfonamides are positively charged under acidic conditions and negatively charged under alkaline conditions (Haller et al., 2002). Acid dissociation constants (pKa) for tetracyclines and sulfonamides are 3.3/7.3/9.1 and 2.5/5.5–7, respectively. Macrolides like ERY and TYL are composed of large lactone rings substituted with hydroxyl, alkyl, and ketone groups. Neutral and amino sugars are bound to the nucleus by the substitution of hydroxyl groups (McArdell et al., 2003). Monensin (MNS), an ionophore, consists of a carboxylic polyether backbone that forms pseudo-macro cyclic complexes with cations (Volmer and Lock, 1998).

The objective of this study was to evaluate the potential for agricultural runoff to contribute antibiotics to surface water bodies by monitoring antibiotic concentrations in runoff from a worst-case scenario. Our hypothesis was that there would be significant differences among antibiotics in the aqueous and sediment concentrations, partitioning of antibiotic losses between runoff and sediment, and pseudo-partitioning coefficients (P-PCs; ratio of sediment concentration to runoff concentration).

MATERIALS AND METHODS

Seven veterinary antibiotics: tetracycline (TC), chlortetracycline (CTC), sulfathiazole (STZ), sulfamethazine (SMZ), erythromycin (ERY), tylosin (TYL), and 90 to 95% monensin sodium salt (MNS) were purchased from Sigma-Aldrich Co. (St. Louis, MO). High performance liquid chromatography-(HPLC) grade methanol (99.9%), analytical-grade formic acid (99%), citric acid monohydrate, sodium phosphate dibasic anhydrous, and disodium ethylene diaminetetraacetic acid (Na$_2$EDTA) were purchased from Sigma-Aldrich Co. (St. Louis, MO). The HLB (hydrophilic lipophillic balance) solid phase extraction (SPE) cartridges (3 mL/60 mg) were purchased from Water Oasis Co. (Milford, MA). Milli-Q water (18.3mΩ) from a Millipore (Billerica, CA) purification system was used when deionized (DI) water was required.

Liang et al. (1998) documented the degradation of tetracycline in methanol solutions stored at room temperature for 6 mo. To avoid degradation, standard solutions (100 μg mL^{-1}) of seven antibiotics (TC, CTC, STZ, SMZ, ERY, TYL, and MNS) were prepared in methanol and stored at 4°C for a maximum of 1 mo. Working solutions, 5 mg L^{-1} and 0.5 mg L^{-1}, were prepared weekly by dilution of the standard solution in methanol.

Experimental Site

The research site was located in north-central Colorado at the Colorado State University Agricultural Research, Development, and Educational Center (ARDEC) north of Fort

Compounds	R$_1$	R$_2$	R$_3$	R$_4$
Tetracycline (TC)	H	OH	CH$_3$	H
Chlortetracycline (CTC)	H	OH	CH$_3$	Cl

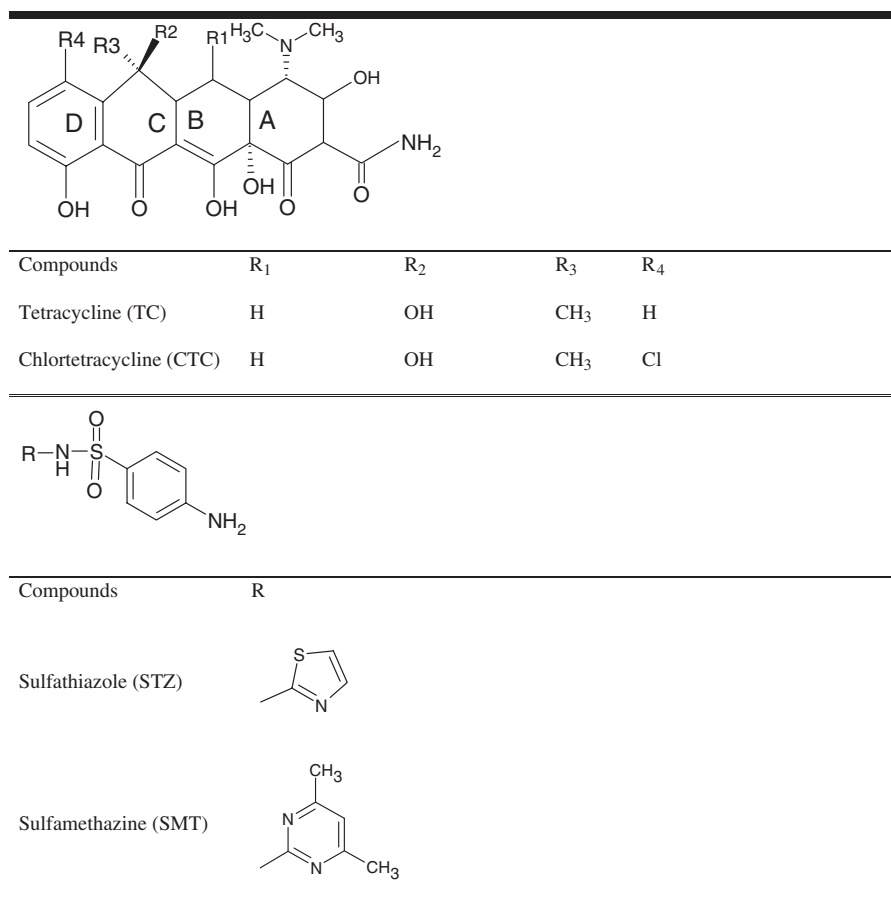| Compounds	R
Sulfathiazole (STZ)	
Sulfamethazine (SMT)	

Fig. 1. Structure of tetracyclines and sulfonamides examined in this study.

Collins, CO (Township 8N, Range 68W, and the SW quarter of section 15). The soil was a Fort Collins sandy clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalf) with a 2% slope. Surface horizon properties were 550 g sand kg^{-1}, 160 g silt kg^{-1}, 290 g clay kg^{-1} (Gee and Or, 2002), pH 7.9 (Thomas, 1996), EC of 1.19 dS m^{-1} (Rhoades, 1996), 18 g OM kg^{-1} (Nelson and Sommers, 1996), cation exchange capacity (CEC) of 24.2 cmoles kg^{-1} (Sumner and Miller, 1996), and 44 g CaCO$_3$ kg^{-1} (Loeppert and Suarez, 1996).

In the 4 yr before this study, sprinkler-irrigated corn (*Zea mays* L.) had been grown for grain under conventional tillage. The site was prepared to simulate typical soil preparation for corn planting. On 24 June 2004 glyphosate was sprayed at 3.45 L ha^{-1}, on 6 July the land was disked twice, on 7 July fertilizer was applied at a rate of 88 kg P$_2$O$_5$ ha^{-1} (0–45–0) and incorporated with a springtooth cultivator followed by 1.3 cm irrigation, on 13 July the area was harrowed, and then a planter (John Deere 7300 Maxemerge) was run through the plots empty to simulate planting conditions.

Antibiotic Application Method

To evaluate a worst-case scenario, antibiotics were applied 1 h before rainfall simulation using a backpack sprayer. This brief period between application and rainfall simulation minimized photo-degradation losses. Based on similar studies of pesticide transport (Leonard, 1990; Potter et al., 2003), we expected that the 1-h delay would maximize antibiotic concentrations in runoff. Kay et al. (2004) found that antibiotic concentrations in tile drainage declined with time after appli-

cation. Kreuzig and Höltge (2005) also reported a high leaching risk for sulfadiazine (a sulfonamide antibiotic) only when antibiotic application was followed immediately with heavy precipitation. On the other hand, Burkhardt et al. (2005) found higher sulfonamide concentrations in runoff from manured grassland when irrigation occurred 3 d after application than when irrigation was applied 1 d after application.

An antibiotic solution was prepared by dissolving seven antibiotics (TC, CTC, STZ, SMT, ERY, TYL, and MNS) in methanol (2.5% volume/volume of final volume) and then bringing the solution to volume with DI H$_2$O to achieve a 1.0 mg L^{-1} concentration for each of the antibiotics. These antibiotics were chosen to represent a variety of classes: tetracyclines (TC, CTC), sulfonamides (STZ, SMT), macrolides (ERY, TYL), and ionophores (MNS) with a range of properties (Table 1). A backpack sprayer with 4 nozzles (#8005 with screen) on a 1.5-m boom was operated at 207 kPa (30 psi), the operator's pace was calibrated using a metronome, and two passes were made to achieve an application rate of 209 mg per plot. This application rate was based on measured levels of antibiotics in 18 hog and dairy lagoons (Carlson et al., 2004) with the goal of applying the approximate amount of antibiotics that would normally be applied when utilizing liquid manure at 220 kg N ha^{-1}, a typical N application rate for corn, assuming 0.6 g N L^{-1} effluent (Davis et al., 2002). We assumed an average concentration of 1 mg L^{-1} for all antibiotics in manure effluent; this value is approximately 90% of the average measured CTC concentration in liquid manure, three times the average measured STZ concentration, and 100 to 1000 times the average concentrations for the other antibiotics (Carlson et al., 2004).
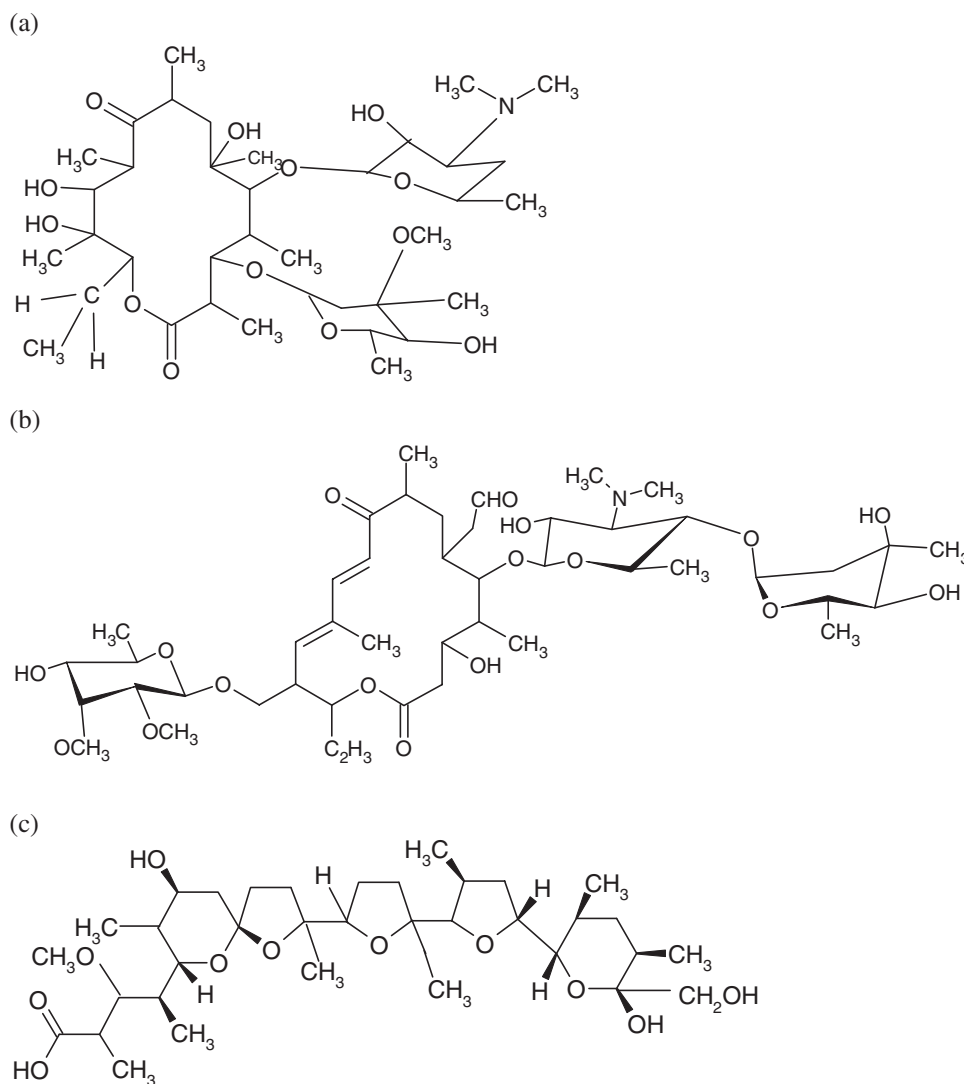
Fig. 2. Structure of (a) erythromycin (ERY), (b) tylosin (TYL), and (c) monensin (MNS).

Filter Targets and Extraction Method

Three filter targets (12.5-cm diameter Baxter Grade 360) were randomly placed on each plot before antibiotic application to measure the actual application rate. Immediately after application, filter targets were collected, wrapped in Al foil, and placed in a plastic bag. Filter targets were taken to the laboratory for analysis. Each target was placed in a 250-mL flask, and 40 mL of methanol were added. Flasks were shaken for 20 min at 400 excursions min^{-1}. Then 5 mL of the extractant was transferred into a 15-mL graduated cylinder. Extracts were concentrated until 50 μL remained, and 70 μL of mobile phase A (0.01 M formic acid) were added in preparation for HPLC analysis as described below.

Rainfall Simulations

Three rainfall simulation plots (6 m^2; 2 m wide by 3 m long) were established within a 0.5-ha area at the ARDEC complex (19 July 2004). The plots were established parallel to the slope (~2%) and row direction, and consisted of two half-beds containing one row each (76-cm row spacing) and a wheel-track middle. A 3-m-wide by 4-m-long area surrounding each 6-m^2 simulator plot was tilled and received antibiotic application identical to the test area to allow soil material to be splashed in all directions. Immediately before simulating rainfall, soil water content was determined gravimetrically (Gardner, 1986) from samples taken from three areas outside of each 6-m^2 rainfall simulation plot. Gravimetric soil water samples averaged 31 (CV = 13%) and 189 (CV = 3%) g water per kg dry soil in 0- to 2- and 2- to 15-cm depth increments, respectively.

Simulated rainfall was applied to each 6-m^2 plot with an oscillating nozzle rainfall simulator (Frauenfeld and Truman, 2004) that used 80150 Veejet nozzles (median drop size = 2.3 mm). The simulator was placed 3 m above each plot. Well water was used in all simulations (pH = 8.0, EC = 0.10 dS m^{-1}). Simulated rainfall was applied in a variable rainfall intensity pattern (Fig. 3) obtained from 15-min natural rainfall data collected at Fort Collins (1975 to 2002) and Byers (1971 to 2002), Colorado (EarthInfo, 2004). These two sites represent all northeastern Colorado climate stations with at least 10 yr of data available. Rainfall during the months of March through August were analyzed to determine the most extreme event occurring during the row-crop growing season over a >30 yr period, and the simulated worst-case rainfall pattern was based on this most extreme rainfall event. Rainfall duration for each simulation was 60 min.

Runoff and sediment yields from each 6-m^2 simulator plot were measured continuously at 5-min intervals during each

Table 1. Characteristics of seven antibiotics used in this study.

Class	Compounds	Usage	Log K_{ow}	Texture	pH	g OC kg^{-1}	K_d	K_{oc}	Reference
				Soil properties					
Tetracyclines	Tetracycline	Cattle and swine†	−1.3‡	Peat	4.5	–	1620		Sithole and Guy (1987)
				Peat	6.1	–	1140		
	Chlortetracycline	Cattle, beef, other	−0.6‡	21¶	7.5	2.9	5706		Sassman and Lee (2005)
				64	3.8	0.5	164973		
				2	5.4	0.5	22		
				82	4.9	0.7	423		
Sulfonamides	Sulfamethazine	Cattle, beef, other	0.28§	Sand	5.2	0.9	1.2	174	Thiele-Bruhn (2003)
				Loamy sand	5.6	2.3	3.1	125	
				Sandy loam	6.3	1.2	2.0	208	
				Silty clay	6.9	1.1	1.0	82	
	Sulfathiazole	Swine	−0.43§	Clay loam	6.2	3.1	3	97	
Macrolides	Tylosin	Swine†	1.63#	Loamy sand	6.1	1.6	128	7990	Rabolle (2000)
				Sand	5.6	1.4	10.8	771	
				Sandy loam	5.6	1.1	62.3	5660	
				Sand	6.3	1.5	8.3	553	
	Erythromycin	Cattle, beef, feedlot, chicken, turkey	3.06††						
Ionophores	Monensin	Cattle, beef	5.4–8.5‡‡						

† From U.S. Food and Drug Administration (2005).
‡ From Thiele-Bruhn (2005).
§ From Adams et al. (2002).
¶ % clay/pH/g OC kg^{-1}.
# From Loke et al. (2002).
†† From Jacobsen et al. (2004).
‡‡ From Thiele-Bruhn (2003).

simulated rainfall event. All of the runoff and sediment was collected at the down-slope end of each 6-m^2 plot in a V-trough collector extending the width of each plot in stainless steel buckets and transferred to 1-L Nalgene (autoclavable) bottles. Bottles were weighed (bottle + water + sediment), dried at 105°C for 24 h, then weighed again (bottle + sediment) to determine runoff and sediment gravimetrically. In addition, a 1-L sample was collected in every 5-min interval for antibiotic analysis.

Sample Pretreatment

Runoff samples were transferred into 50-mL centrifuge tubes for separation of the aqueous and solid phases before antibiotic analysis. Samples were centrifuged at 1917 g for 30 min. The aqueous phase was filtered through a 0.2-μm pore size glass fiber filter and decanted to another 1-L sampling bottle. The solid phase was collected in plastic bags. Both aqueous and sediment samples were kept in a cooler during the experiment and transferred to a refrigerator the same day. Solid phase samples were air-dried in a dark room to prevent photodegradation.

Sample Extraction

Aqueous samples were cleaned and enriched using solid phase extraction (SPE) as described by Lindsey et al. (2001)
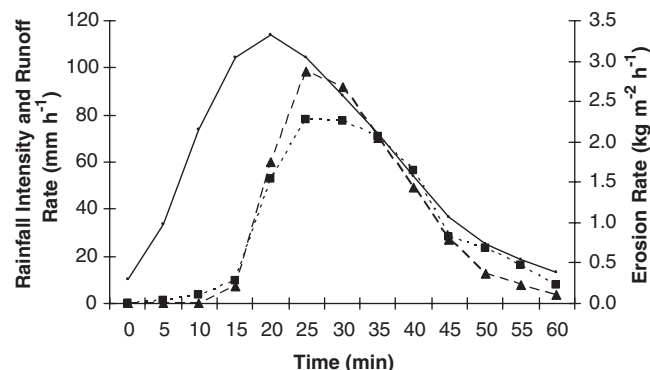

Fig. 3. Rainfall intensity (●) and average runoff (▲) and erosion rates (■) for a variable-intensity simulated rainstorm.

and Yang and Carlson (2003). A 3 mL/60 mg capacity HLB cartridge was used to extract the applied compounds. The HLB cartridge was known to have a durable capacity for both hydrophilic and hydrophobic compounds and was also suitable for TC to avoid the irreversible binding of this compound to silanol groups. One hundred twenty mL of sample was carefully measured into a flask, and the pH was adjusted to 2.3 to 2.5 for TC, CTC, STZ, and SMZ using 40% (volume/volume) H$_2$SO$_4$ solution. For ERY and TYL, the pH was adjusted to 5.0. This was followed by the addition of 0.5 mL of 0.02 M Na$_2$EDTA to chelate any metals in the sample. No pH adjustment or addition of Na$_2$EDTA was used for analysis of MNS.

Before loading the sample, the HLB cartridge was preconditioned with 3 mL of methanol, then 3 mL of 0.5 M HCl, followed by 3 mL of DI water. Methanol and 0.5 M HCl were used to activate the cartridge and to remove any metal residuals in the cartridge, respectively, and DI water was used to remove impurities in the cartridge. The sample was loaded under a 276 kPa (40 psi) vacuum with a flow rate of approximately 2 mL min^{-1}. After loading the sample, the SPE cartridge was rinsed with 9 mL of water to remove any impurities and excess Na$_2$EDTA. For quantification of the analytes in the samples, 50 μL of the internal standard, simatone (0.24 mg L^{-1} stock solution), was added to a 15-mL graduated cylinder before adding the extract sample to the cartridge. Simatone was chosen as the internal standard based on a previous study and has shown good response to our experimental conditions (Lindsey et al., 2001). Five mL of methanol was used to extract the loaded sample into the cylinder, and then the sample was concentrated with evaporation in a 50°C water bath under a gentle N gas flow to prevent oxidation. Samples were concentrated to a volume of 50 μL, and 70 μL of mobile-phase solution (0.01 M formic acid) were added. Samples were transferred into amber vials equipped with 150-μL glass inserts for high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis.

For sediment samples, two stages of analytical preparation were conducted. The first stage was to extract compounds from the solid phase into the liquid phase. McIlvain buffer solution (pH 4.0) was used for TC, CTC, STZ, and SMZ, and ammonium hydroxide buffer solution (1 M, pH 10.0) was used

for ERY, TYL, and MNS to extract each of the compounds from the solid phase into the liquid phase. McIlvain buffer solution was prepared according to USDA (United States Department of Agriculture) guidelines (USDA, 2003). Ammonium hydroxide buffer solution (1 M) was titrated with formic acid to adjust the pH to 10.0.

One g of sediment sample was weighed with a precision of 0.001 g and transferred into 40-mL vials. Twenty mL of McIlvain buffer solution or ammonium hydroxide buffer solution were added followed by 200 μL of 0.02 M Na$_2$EDTA. The sample was vigorously mixed in the parallel shaker (Model No-4626, Labline instrument) for 20 min at 400 excursions min^{-1}. The sample was then centrifuged at 1434 g (IEC Clinical Centrifuge, International Equipment Co., Needham Heights, MA) for 15 min followed by filtration using 0.2-μm glass fiber filters. The filtered sample was decanted into another 40-mL vial and kept in the refrigerator. The extraction was repeated once in the same manner as described above, and supernatants were combined for the SPE cleanup procedure.

The second stage of the analytical preparation was cleanup and concentration of the sample. The SPE condition for this cleanup and concentration step was the same as the aqueous sample except the addition of 0.5 M HCl was omitted.

High Performance Liquid Chromatography-Tandem Mass Spectrometry

The HPLC analyses were performed using an HP 1100 Series Liquid Chromatograph (Agilent, Palo Alto, CA) equipped with an Agilent 1100 Series Thermostatted Auto Sampler and a variable-wavelength UV detector. An XTerra MS C$_{18}$ (Waters, Milliford, MA) 2.1 $\times$ 50 mm (2.5-μm pore size, end-capped) reversed-phase column was used to analyze the standards and samples. A C$_{18}$ guard column (Phenomenex, Torrence, CA, USA) was used to filter any particulates from the sample.

A combination of three mobile phases was used depending on the antibiotic group being measured. Mobile phase A was composed of 0.01 M formic acid (pH 2.74), mobile phase B was 9.5 M acetonitrile mixed with 0.01 M formic acid, and mobile phase C was pure methanol. The column temperature was set to 15°C for tetracycline and sulfonamide measurement, and the gradient for tetracycline was ramped from 96% mobile phase A and 4% mobile phase B to 70% mobile phase A and 30% mobile phase B for 19 min and then 96% mobile phase A and 4% mobile phase B for 1 min. The gradient for measurement of sulfonamides was programmed for 21 min with the same conditions as for tetracyclines. For macrolides, the column temperature was set to 45°C, and the gradient was programmed to ramp from 80% mobile phase A and 20% mobile phase B to 65% mobile phase A and 35% mobile phase B for 14 min and then to the original condition for 1 min. Ten min of post-run time was used to equilibrate the column. The flow rate was set at 0.32 mL min^{-1}, and the injection volume was 20 μL for tetracyclines, sulfonamides, and macrolides.

For ionophore polyethers, the column was maintained at 15°C with a flow rate of 0.25 mL min^{-1}. The gradient was ramped from 50% mobile phase A and 50% mobile phase C to 10% mobile phase A and 90% mobile phase C in the first minute and held isocratic for 19 min. The injection volume was 20 μL, and a 10-min post-run period was allowed between each analysis to re-equilibrate the column.

A ThermoFinnigan LCQ Duo ion-trap mass spectrometer (ThermoQuest, Woburn, MA) equipped with a heated capillary interface and electrospray ionization was used to perform the mass spectrometric analysis. Ten-μM standard solutions of tetracyclines, sulfonamides, macrolides, and ionophore polyethers were made in DI water and injected using the LCQ Duo syringe pump at a flow rate of 5 μL min^{-1} to optimize the mass spectrometry parameters as needed. Nitrogen gas was used for drying and nebulizing. The spray voltage was set to 4.5 kV, and the capillary voltage autotuned to 21 V. The capillary temperature was set to 165°C, and the instrument was operated in the positive ion mode. The sheath gas flow rate was optimized at 40 units, and the auxiliary gas was turned off. The precursor mass and product ion optimized tandem mass spectrometry parameters are summarized in Table 2, and the recoveries and limits of quantification (LOQ) for these analyses are shown in Table 3.

Statistical Analysis

The P-PCs were calculated as the ratio of antibiotic concentration on the sediment to the antibiotic concentration in the runoff. When a sample had a concentration below the LOQ, then the midpoint between zero and the LOQ was utilized for that value, as is common practice in medical (Olsen et al., 2003) and environmental (Gelsleichter et al., 2005) research. For statistical analysis the concentrations, absolute losses, relative losses, and P-PCs were transformed to their natural logarithms to achieve normal distributions.

A mixed model (SAS, 2002) was utilized to complete an analysis of variance evaluating the main effects of antibiotic, replicate, and sampling time on antibiotic concentrations and P-PCs (repeated measures). If the main effect was significant ($p < 0.05$), then least significant differences (LSDs) were used to compare the least-square means of the antibiotic concentrations and P-PCs. In addition, a general linear model was run within each interval to evaluate differences among antibiotic concentrations and P-PCs.

The absolute antibiotic losses and the relative losses (% of applied and % associated with sediment) were analyzed in a similar manner to concentrations and P-PCs using a mixed model; however, for these parameters, there were no repeated measures, just one data point per plot.

RESULTS AND DISCUSSION

Average antibiotic application rates as measured on filter targets ranged from 197 mg plot^{-1} for ERY to 308 mg plot^{-1} for TC (Table 4). Our application rate goal was 209 mg plot^{-1}; however, the operator of the backpack sprayer noticed that the sprayer was almost empty after spraying the final plot, implying an accidental application rate about 26% higher than intended (263 mg plot^{-1}). Coefficients of variation from three targets within replicate plots ranged from 1 (SMT rep 2) to 26% (ERY rep 1) and averaged 14%. Coefficients of variation across replicates were all $\leq$10%.

Simulated rainfall intensity increased rapidly to a peak of 115 mm h^{-1} at 17 to 18 min into the simulation and then

Table 2. Optimized HPLC tandem mass spectrometry parameters (fragment ions in italic were used to quantify the concentration of sample).

Compound	Precursor mass [M+H]$^+$	Fragment ions	Isolation width	Collision energy
	m/z			%
Tetracycline	445	*427*	2.0	26
Chlortetracycline	479	*462*, 444	2.0	32
Sulfathiazole	256	*156*	2.0	32
Sulfamethazine	279	156, *204*	2.0	38
Erythromycin-H$_2$O	716	522, *558*	3.0	26
Tylosin	916	*772*	3.0	30
Monensin	693	*675*	2.0	28

Table 3. Recovery and limits of quantification (LOQ) for antibiotic analytical methodology. TC = tetracycline, CTC = chlortetracycline, STZ = sulfathiazole, SMT = sulfamethazine, ERY = erythromycin, TYL = tylosin, and MNS = monensin.

| | Water[†] | | | | | Sediment | | | | |
| | Recovery | | | LOQ[‡] | | Recovery | | | LOQ | |
Antibiotic	0.1 μg L^{-1}	1 μg L^{-1}	5 μg L^{-1}	S/N = 3§	Statistical method	1 μg kg^{-1}	30 μg kg^{-1}	90 μg kg^{-1}	S/N = 3	Statistical method
	%			μg L^{-1}		%			μg kg^{-1}	
TC	106	109	104	0.01	0.02	82	90	91	1.0	2.3
CTC	104	124	107	0.01	0.02	90	69	64	1.0	2.3
STZ	77	101	92	0.01	0.01	108	106	78	1.0	1.8
SMT	108	109	96	0.01	0.01	92	105	80	1.0	1.8
ERY	102	101	103	0.01	0.01	127	128	101	1.0	0.6
TYL	103	89	114	0.01	0.01	79	74	77	1.0	0.6
MNS	114	124	106	0.001	0.001	71	71	105	1.0	0.4

† Three different concentrations were examined for water and sediment, and the range of relative standard deviation was from 1 to 11% for water and 13 to 31% for sediment.

‡ LOQ was calculated using the standard deviation of the sample spiked with 0.01 μg L^{-1} and 1 μg kg^{-1} and applying the student t-variate with a 95% confidence interval.

§ S/N (signal to noise ratio) was determined by HPLC/MS/MS, measuring the lowest calibration points and following the statistical method of Zhu et al. (2001).

declined gradually through the rest of the 1-h rainfall event (Fig. 3). Runoff and soil loss rates followed a similar pattern, with peaks a few minutes later during the 20- to 25-min period, followed by a decline.

Runoff and sediment concentrations averaged over time were significantly different among antibiotics (Table 5). Aqueous concentrations were greatest for MNS and least for the tetracyclines and TYL. On the other hand, sediment concentrations were highest for ERY, followed by MNS and then TYL. The average sediment concentration for SMZ was below the LOQ and, therefore, cannot be distinguished from zero.

In addition, there were significant differences in antibiotic concentrations with time during the rainfall simulation event (Fig. 4). Aqueous concentrations revealed significant differences only at 5- and 20-min sampling times. At 5 min, SMT had the highest aqueous concentration followed by MNS and STZ; the tetracyclines and macrolides had the lowest aqueous concentrations. At 20 min, MNS had the highest aqueous concentration, followed by SMT; the tetracyclines and TYL had the lowest concentrations. The TC concentrations were below the LOQ of 0.01 μg L^{-1} after 30 min, and CTC concentrations also fell below the LOQ after 50 min.

Table 4. Mean application rates and standard deviations (with coefficients of variation in parentheses) as measured on filter paper targets.

| | Application rates[†] | | | |
Antibiotic	Plot 1‡	Plot 2	Plot 3	Plot mean
	mg plot^{-1}			
Tetracycline	316 ± 32 (10)	296 ± 23 (8)	313 ± 16 (5)	308 ± 10 (3)
Chlortetracycline	245 ± 51 (21)	219 ± 15 (7)	247 ± 28 (12)	237 ± 16 (7)
Sulfathiazole	274 ± 46 (17)	251 ± 48 (19)	278 ± 28 (10)	267 ± 15 (6)
Sulfamethazine	258 ± 59 (23)	224 ± 2.7 (1)	258 ± 30 (11)	247 ± 20 (8)
Erythromycin	206 ± 55 (26)	175 ± 34 (19)	209 ± 30 (14)	197 ± 19 (10)
Tylosin	277 ± 65 (24)	241 ± 32 (13)	275 ± 32 (12)	264 ± 20 (8)
Monensin	282 ± 39 (14)	252 ± 34 (13)	280 ± 17 (6)	271 ± 17 (6)

† Target rate for each compound was 209 mg plot^{-1}.

‡ There were three spray targets per plot.

Sediment concentrations were significantly different from 10 to 20 min and again at 60 min (Fig. 4). During the 10 to 20 min interval, ERY consistently had the highest sediment concentrations followed by MNS and TYL. At 60 min, the sediment concentration of TYL was greater than all others. The LOQ for sediment concentrations was 1 μg kg^{-1}, and concentrations were below this level for CTC and SMT after 20 min, TC after 30 min, and STZ and MNS after 45 min.

There were also significant differences among antibiotics in the absolute losses measured in runoff and erosion (Table 6). The MNS was lost in the greatest amounts, followed by ERY, and TC and CTC were lost in the least amounts. The same differences were found when relative loss was calculated as a percentage of the measured amounts applied. Note that all of the antibiotics lost <0.1% of the total applied in this 1-h extreme rainfall event only 1 h after antibiotic application. Kay et al. (2005c) also reported losses <0.1% for OTC and sulfachloropyridazine and undetectable losses of TYL from their plot without a tramline (comparable to our plots). Kreuzig et al. (2005) and Burkhardt et al. (2005) measured slightly higher runoff losses (≤2.5%) for sulfonamides applied to cropland.

The relative loss associated with sediment is a helpful indicator for evaluating management practices to reduce off-site transport. For example, when the relative sediment loss is high, erosion control practices would be expected to be effective in minimizing off-site transport.

Table 5. Average antibiotic concentrations in aqueous and sediment portions of runoff collected during a 1-h simulated rainfall event (n = 36).

| | Concentration | |
Antibiotic	Aqueous	Sediment
	μg L^{-1}	μg kg^{-1}
Tetracycline	0.03d†	1.3de
Chlortetracycline	0.04d	1.5de
Sulfathiazole	0.33bc	2.0d
Sulfamethazine	0.58b	0.6e
Erythromycin	0.21c	17.0a
Tylosin	0.09d	8.0c
Monensin	1.20a	10.5b

† Antibiotics with a common letter are not significantly different by least significant differences (LSD) (p < 0.05) within each column.
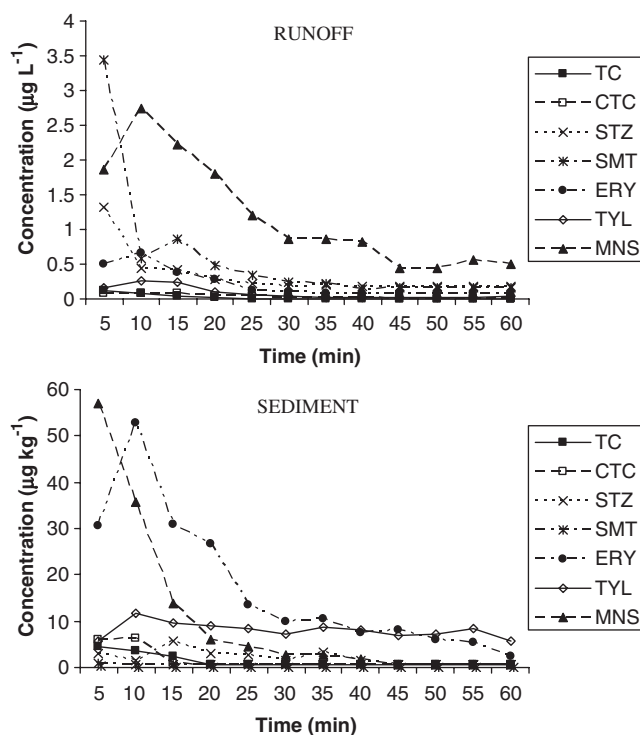
Fig. 4. Average antibiotic concentrations in runoff aqueous phase (top) and sediment solid phase (bottom). TC = tetracycline, CTC = chlortetracycline, STZ = sulfathiazole, SMT = sulfamethazine, ERY = erythromycin, TYL = tylosin, and MNS = monensin.

Antibiotics were significantly different from one another in their relative loss associated with sediment (Table 6). The TC, ERY, and TYL had the highest relative losses associated with sediment, and MNS and SMT had the lowest losses. Therefore, we would expect that erosion control practices that reduce sediment loss would be most effective at reducing TC, ERY, and TYL transport off-field, but that these practices would not be effective for the SMT or MNS.

In general, the values reported here for relative losses associated with sediment tend to be higher than those in the literature, probably due to the field condition being recently tilled with no plant cover, a worst-case scenario. Kreuzig et al. (2005) stated that the sulfonamides lost in runoff occurred predominantly in the aqueous phase, and losses via suspended matter were of low relevance

Table 6. Cumulative antibiotic losses in runoff expressed as absolute and relative values.

Antibiotic	Absolute loss	Relative loss as fraction of applied‡	Relative loss associated with sediment
	mg plot^{-1}	%	
Tetracycline	0.005e†	0.002e	65a
Chlortetracycline	0.009d	0.004d	34b
Sulfathiazole	0.056c	0.021c	23c
Sulfamethazine	0.059c	0.024c	5e
Erythromycin	0.096b	0.049b	74a
Tylosin	0.057c	0.022c	77a
Monensin	0.219a	0.081a	9d

† Antibiotics with a common letter are not significantly different by least significant differences (LSD) ($p < 0.05$) within each column.
‡ Relative losses were calculated on a plot-by-plot basis using the actual measured application rates reported in Table 4.

(<1% of absolute loss). Kay et al. (2005c) also reported particulate losses of a sulfonamide to be only 1.1% of absolute loss, but the field was in wheat stubble, thus reducing erosion potential. We evaluated different sulfonamides than Kreuzig et al. (2005) or Kay et al. (2005c) in our study on bare, tilled soil and found the relative losses associated with sediment to be 5% and 23% for SMT and STZ, respectively (Table 6).

Few comparable data exist for tetracyclines. Kay et al. (2005c) measured a relative loss for OTC associated with sediment to be 7.4% on a field in wheat stubble. We evaluated TC and CTC on bare ground and found much higher relative losses, 65% and 34%, respectively. The ground cover and kind of soil have critical impacts on runoff and soil loss. In addition, antibiotics from the same class can vary considerably in their chemical properties.

Although concentration maxima tended to occur within the first 5 to 10 min of the rainfall simulations (Fig. 4), the maximum rates of mass loss occurred later (Fig. 5) due to the occurrence of peak runoff and soil loss values at 20 to 25 min (Fig. 3). The aqueous loss rates peaked at 20 min for TC, ERY, TYL, and MNS, and at 25 min for CTC, STZ, and SMT (Fig. 5). The maximum sediment loss rates occurred at 25 min for all of the antibiotics measured except for ERY, which peaked at 20 min.

Antibiotics also showed significant differences in their P-PCs (Table 7). In particular, TYL had the highest P-PC as compared to STZ and ERY, the other antibiotics whose P-PCs did not change with time. The P-PC values changed significantly with time for some of the
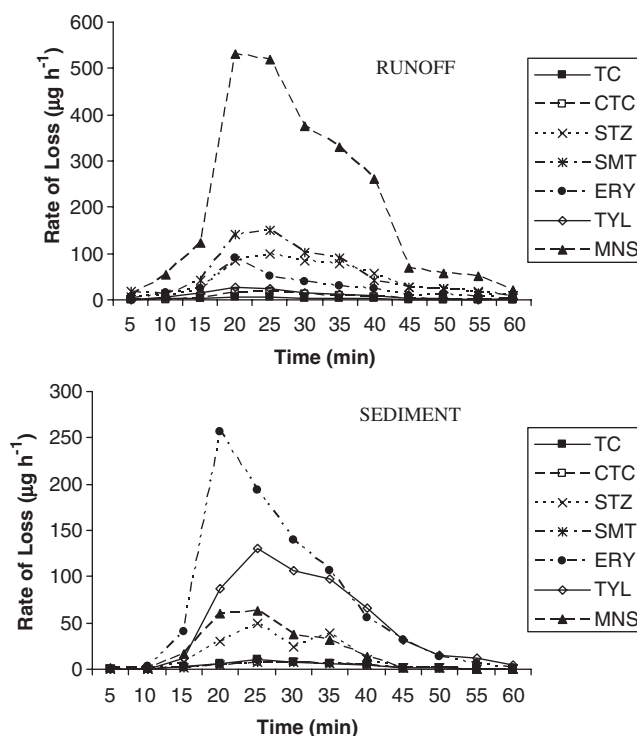

Fig. 5. Average rate of mass loss as a function of time in runoff aqueous phase (top) and sediment solid phase (bottom). TC = tetracycline, CTC = chlortetracycline, STZ = sulfathiazole, SMT = sulfamethazine, ERY = erythromycin, TYL = tylosin, and MNS = monensin.

Table 7. Average pseudo-partitioning coefficients as measured in small plot simulated runoff samples.

Antibiotic	Pseudo-partitioning coefficient[†]
	L kg^{-1}
Tetracycline	84[‡]
Chlortetracycline	56
Sulfathiazole	7b
Sulfamethazine	2
Erythromycin	116b
Tylosin	1061a
Monensin	6

[†] The pseudo-partitioning coefficients were calculated on a plot-by-plot basis using the actual measured concentrations for each interval ($n = 36$).

[‡] Antibiotics with a common letter are not significantly different by least significant differences (LSD) ($p < 0.05$). Only those pseudo-partitioning coefficients that did not change with time were compared by LSD.

measured compounds (Fig. 6). For MNS, the P-PC was highest in the first 5 min of the simulation, whereas SMT and TC peaked near the end of the run at 40 to 60 and 25 to 60 min, respectively. The CTC had maximum P-PC values both early (5 to 10 min) and late (45 to 60 min) in the rainfall event. The average P-PC for TYL was an order of magnitude greater than all of the P-PCs (by time increment) for those antibiotics that did change with time.

In addition, time-averaged P-PCs (Table 7) differed from published K_d values generated in laboratory experiments (Table 1). The P-PC for TC was less than lab-determined K_d values, but the P-PC for TYL was greater than reported K_d values. The CTC, STZ, and SMT had measured P-PCs similar to lab-determined K_d values, and therefore, these antibiotics were apparently near equilibrium, whereas TC and TYL were not. No published K_d values were found for ERY or MNS.

This study evaluated the transport of antibiotics applied directly to soil in an aqueous matrix. Burkhardt et al. (2005) demonstrated that pig slurry application to soil increased runoff volume, apparently due to surface sealing and reduced infiltration that resulted in increased sulfonamide losses compared to a control that did not receive slurry. Davis-Carter and Burgoa (1993) revealed a similar impact on atrazine runoff from soil-applied dairy effluent. In addition, Burkhardt et al. (2005) found that manure application also affected the relative mobility of
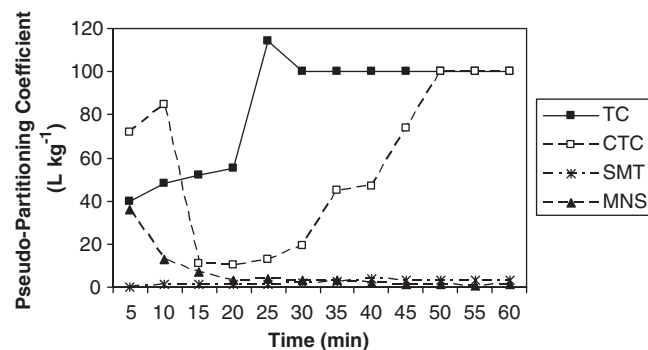

Fig. 6. Average pseudo-partitioning coefficient as a function of time for TC, CTC, SMT, and MNS. Time had no impact on the pseudo-partitioning coefficients for STZ, ERY, and TYL, and therefore they are not shown. TC = tetracycline, CTC = chlortetracycline, SMT = sulfamethazine, MNS = monensin, STZ = sulfathiazole, ERY = erythromycin, and TYL = tylosin.

the sulfonamides. Hence, the manure matrix has both physical and chemical effects on environmental fate. Kreuzig and Höltge (2005) also reported that the manure matrix effect may have an important impact on sulfonamide mobility in soils. On the other hand, Kay et al. (2005b) concluded that pig slurry had no impact on OTC leaching. Therefore, the importance of the matrix effect may vary depending on antibiotic chemistry. In the future, antibiotic transport should be monitored from fields receiving manure applications to evaluate the impact of the manure matrix on transport characteristics.

CONCLUSION

This field study demonstrates that antibiotics have different transport characteristics. Antibiotic type significantly impacted the partitioning of antibiotic losses between runoff and sediment. The MNS had the highest concentration in runoff and the second highest concentration on the sediment (Table 5), resulting in the highest absolute loss of all the antibiotics tested (Table 6). However, in spite of relatively high sediment concentration, the relative loss associated with sediment transport was <10% for MNS (Table 6) since aqueous concentrations were also high. ERY had the highest concentrations in sediment (Table 5), relatively high total loss (second to MNS), and was among the antibiotics with the highest relative loss associated with sediment (>50%) (Table 6). The TYL, the other macrolide studied, also had >50% relative loss associated with sediment and relatively high sediment concentration (Table 5), and its P-PC was the highest of those measured (Table 7). On the other hand, TYL was among those antibiotics with the lowest aqueous concentrations in runoff (Table 5). The tetracyclines (TC and CTC) also had very low aqueous concentrations, and had the lowest absolute losses compared to the other antibiotics evaluated (Table 6).

If agricultural antibiotic runoff is proven to result in the development of antibiotic resistant genes or toxicity to aquatic organisms, then management practices will be needed to reduce antibiotic runoff. Erosion control practices are predicted to result in reduced loss of TC, ERY, and TYL to surface water bodies, but other tested antibiotics probably will not respond to soil conservation practices alone. Other methods will need to be developed in addition to erosion control practices to reduce the off-site transport of the other tested antibiotics to the environment.

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